

LISTING OF THE CLAIMS

1. (Currently amended) A microfluidic device ~~for processing a cell-containing microdroplet~~, comprising:
 - ~~a channel;~~
 - a lysing zone connected to the channel, module configured to receive a cell-containing microdroplet;
 - ~~an actuator connected to the channel only~~ located upstream of the lysing zone ~~module~~ and configured to create a difference between an upstream pressure and a downstream pressure acting on the cell-containing microdroplet to move the microdroplet ~~at least partially into~~ towards the lysing zone, ~~module;~~
 - ~~a vent connected to the channel~~ vented positioning element located upstream of the lysing zone ~~module~~ and downstream of the actuator, wherein the vented positioning element is configured to ~~reduce the pressure difference to~~ stop the cell-containing microdroplet in a lysing position with respect to the lysing zone, ~~module;~~ and
 - a lysing mechanism within the lysing module, configured to release intracellular material from cells within the cell-containing microdroplet ~~stopped within in~~ the lysing position with respect to the lysing zone module.
2. (Original) The microfluidic device of claim 1, wherein the cell-containing microdroplet comprises cells entrained in a liquid.
3. (Currently amended) The microfluidic device of claim ~~[[2,]]~~ 1, wherein ~~actuation of~~ the lysing mechanism is configured to subject ~~subjects cells~~ the cell-containing microdroplet in the lysing zone ~~module~~ to an electric field sufficient to release the intracellular material from the cells.
4. (Currently amended) The microfluidic device of claim ~~[[2,]]~~ 1, wherein ~~actuation of~~ the lysing mechanism ~~subjects substantially all of the cells in the lysing zone to the electric field~~ is configured to prepare a lysed microdroplet comprising the ~~released~~ intracellular material.
5. (Cancelled)

6. (Cancelled)

7. (Cancelled)

8. (Cancelled)

7 ~~9~~. (Previously presented) The microfluidic device of claim ~~13~~⁵, wherein the reduced wetting material is hydrophobic.

10. (Cancelled)

11. (Cancelled)

~~12~~. (Currently amended) The microfluidic device of claim 1, wherein the ~~[[vent]]~~ vented positioning element is configured to position a ~~downstream~~ portion of the cell-containing microdroplet downstream of the vented positioning element in the lysing position.

5 ~~13~~. (Currently amended) The microfluidic device of claim ~~12~~¹, wherein the vented positioning element comprises ~~comprising~~ a reduced wetting material configured to prevent the microdroplet from ~~contacting~~ flowing into the vent.

6 ~~14~~. (Currently amended) The microfluidic device of claim ~~13~~⁵, wherein the vented positioning element further comprising ~~comprises~~ a valve configured to selectively obstruct or allow passage of gas between the reduced wetting material and the vent.

15. (Cancelled)

15 ~~16~~. (Currently amended) A microfluidic device ~~for processing a cell-containing fluid,~~ comprising:
a channel;
a lysing zone ~~connected to the channel~~ module configured to receive a microdroplet of cell-containing sample;

a lysing mechanism within the lysing module, configured to release intracellular contents from cells in the microdroplet of cell-containing sample within the lysing zone module;

a first gas actuator ~~connected to the channel only~~ situated upstream of the lysing zone module and configured to move ~~an amount of a~~ the microdroplet of cell-containing fluid sample downstream ~~at least partially into~~ to overlap the lysing zone module;

a positioning element located downstream of the lysing zone module and configured to inhibit downstream movement of the cell containing fluid sample, thereby positioning at least some of the cell containing fluid sample in a lysing position with respect to the lysing zone module; and

a second gas actuator disposed upstream from the ~~positioning element~~ lysing module but downstream from the first actuator, to provide a gas pressure sufficient ~~[[to]]~~ to:

(a) prepare a lysed microdroplet comprising intracellular contents released from cells of the cell-containing fluid sample within the lysing zone module, the microdroplet having a length ~~substantially~~ equal to a distance between the second gas actuator and the positioning element and (b) move the lysed microdroplet downstream of the lysing zone module and past the positioning element.

17. (Cancelled)

16 ~~18~~. (Currently amended) The microfluidic device of claim ~~[[17,]]~~ ¹⁵ ~~16~~, wherein ~~actuation~~ of the lysing mechanism ~~subjects~~ is configured to subject at least some cells in the lysing zone module to an electric field sufficient to release the intracellular contents of the cells.

17 ~~16~~. (Currently amended) The microfluidic device of claim ~~18~~ ¹⁶, wherein the lysed microdroplet is essentially free of cells that have not been subjected to the electric field.

20. (Cancelled)

18 ~~21~~. (Currently amended) The microfluidic device of claim ~~17~~ ¹⁵, wherein the distance between the gas actuator and the positioning element is ~~configured~~ such that the lysed microdroplet comprises less than about 90 percent of the ~~amount~~ microdroplet of the cell-containing fluid sample.

21¹⁵/~~21~~. (Currently amended) The microfluidic device of claim ~~[[17,]] 16~~¹⁵, wherein the device comprises a substrate, and wherein the lysing ~~zone module, and~~ first gas actuator, second gas actuator, and positioning element, are integral with the substrate.

22²¹/~~22~~. (Currently amended) The microfluidic device of claim ~~22~~²¹, wherein the first gas actuator comprises a heat source configured to heat an amount of gas thereby increasing a pressure of the gas.

24. (Cancelled)

19¹⁵/~~26~~. (Currently amended) The microfluidic device of claim ~~[[17,]] 16~~¹⁵, wherein the positioning element increases a surface tension of a downstream portion of the cell-containing fluid sample ~~[[to]]~~ and is thereby configured to inhibit downstream movement of the cell-containing fluid sample.

20¹⁵/~~26~~. (Currently amended) The microfluidic device of claim ~~[[17,]] 16~~¹⁵, wherein the device comprises a vent configured to substantially equalize a gas pressure upstream of the cell-containing fluid sample with a gas pressure downstream of the cell-containing fluid sample when the cell-containing fluid sample is in the lysing position ~~[[to]]~~ and thereby to inhibit downstream movement of the cell-containing fluid sample downstream from the lysing position.

~~21~~¹⁵. (Currently amended) A ~~microfluidic~~ method for ~~processing~~ lysing a cell-containing ~~liquid~~ microdroplet in a microfluidic device, comprising:
propelling ~~[[a]]~~ the microdroplet toward a lysing mechanism of the microfluidic device by increasing a gas pressure upstream of the ~~microdroplet, microdroplet;~~ venting gas from upstream of the microdroplet to reduce the ~~upstream gas~~ pressure upstream and to stop the cell-containing microdroplet in a lysing position with respect to ~~[[a]]~~ the lysing mechanism ~~of a microfluidic device;~~ and actuating ~~[[a]]~~ the lysing mechanism to release intracellular material from cells of the ~~stopped~~ cell-containing microdroplet in the lysing position.

~~28.~~ (Currently amended) The ~~microfluidic~~ method of claim 27, further comprising increasing a surface tension of a downstream surface of the microdroplet.

~~29.~~ (Currently amended) The ~~microfluidic~~ method of claim 28, wherein the increasing comprises contacting the downstream surface of the microdroplet with a hydrophobic material.

~~30.~~ (Currently amended) The ~~microfluidic~~ method of claim 28, wherein the increasing comprises increasing a radius of curvature of the microdroplet.

31. (Cancelled)

~~32.~~ (Currently amended) The ~~microfluidic~~ method of claim 27, wherein the actuating ~~step~~ comprises subjecting ~~cells of the~~ cell-containing microdroplet to an electric field sufficient to release intracellular contents from the cells.

24 ~~33.~~ (Currently amended) A ~~microfluidic~~ method for ~~processing~~ lysing a microdroplet of cell-containing liquid, comprising:

introducing the microdroplet of cell-containing liquid to a lysing ~~zone~~ module of a microfluidic device;

inhibiting ~~liquid of the microdroplet of~~ cell-containing liquid from ~~exiting~~ moving downstream from the lysing zone, module; then

actuating ~~[[the]]~~ a lysing mechanism to release intracellular contents from cells of the cell-containing liquid within the lysing ~~zone~~ module; and

~~then~~ providing a gas pressure sufficient to separate a first portion of the microdroplet of cell-containing liquid located within the lysing ~~zone~~ module from a second portion of the microdroplet of cell-containing liquid located upstream of the lysing module zone to prepare thereby preparing a lysed microdroplet comprising intracellular contents released from cells of the cell-containing liquid within the lysing ~~zone~~ module.

34. (Cancelled)

28 ~~35~~. (Currently amended) The ~~microfluidic~~²⁴ method of claim ~~35~~²⁴, wherein actuating the lysing mechanism subjects at least some cells within the lysing ~~zone~~ module to an electric field sufficient to release the intracellular contents of the cells.

29 ~~36~~. (Currently amended) The ~~microfluidic device~~²⁸ method of claim ~~36~~²⁸, wherein the microdroplet is essentially free of cells that have not been subjected to the electric field.

25 ~~37~~. (Currently amended) The ~~microfluidic device~~²⁴ method of claim ~~37~~²⁴, wherein the ~~step of providing the gas pressure moves the lysed microdroplet to a location downstream of the lysing zone~~ module.

38. (Cancelled)

8 ~~39~~. (Currently amended) ~~[[A]] The microfluidic device of claim 1, for processing a cell-containing sample material, the device further comprising:~~
a ~~sample passage;~~
a ~~lysing zone in communication with the sample passage;~~
a ~~first gas actuator to move an amount of cell-containing sample liquid along the sample passage toward the lysing zone;~~
a ~~second gas actuator to move only a portion of the amount of the cell-containing sample liquid downstream of the lysing zone upon lysis of cells of the cell-containing sample liquid; and~~
a plurality of valves, at least one of the valves located upstream of the lysing ~~zone~~ module and at least one of the valves located downstream of the lysing ~~zone~~ module, ~~wherein, wherein~~ the valves, when in a closed state, inhibit the passage of material between the lysing ~~zone~~ module and other portions of the microfluidic device.

~~40~~. (Currently amended) A method for lysing cells, the method comprising:
moving a microdroplet of cell-containing liquid within a microfluidic device in response to a change in a pressure of a gas, the microfluidic device comprising a lysing ~~zone~~ module, a first passage actuator upstream of the lysing ~~zone~~ module, and a second actuator located between the first actuator and passage downstream of the

lysing zone module, the microdroplet of cell-containing liquid being moved ~~along the first passage and~~ into the lysing zone module;
inhibiting liquid the microdroplet of the cell-containing liquid from moving downstream of the lysing zone module ~~along the second passage~~; and
~~after inhibiting downstream movement of the liquid~~, lysing cells of the microdroplet of cell-containing liquid within the lysing zone module.

- 26 ²⁴ 41. (Currently amended) The method of claim ~~[[40,]] 33~~²⁴, wherein the inhibiting comprises equalizing a pressure acting on the cell-containing liquid to prevent the cell-containing liquid from moving downstream of the lysing chamber, at least some of the cell-containing liquid stopping within the lysing zone module.
- 27 ²⁴ 42. (Currently amended) The method of claim ~~[[40,]] 33~~²⁴, wherein the inhibiting comprises contacting a downstream boundary of the cell-containing liquid with a reduced wetting material disposed within the second passage.
- 30 ²⁴ 43. (Currently amended) The method of claim ~~[[40,]] 33~~²⁴ further comprising, after lysing cells of the cell-containing liquid, actuating a gas actuator to separate a first portion of the cell-containing fluid from a second portion of the cell containing fluid.
- 31 ³⁰ 44. (Currently amended) The method of claim ~~43~~³⁰, wherein the actuating the gas actuator additionally moves the first portion of the cell-containing fluid along the downstream passage.
- 9 ⁹ 45. (New) The device of claim 1, wherein the lysing mechanism comprises electrodes which are electrically connected to a pulse circuit.
- 10 ⁹ 46. (New) The device of claim ~~46~~⁹, wherein the pulse circuit has a configuration shown in FIG. 14.
- 11 ⁹ 47. (New) The device of claim 1, wherein the actuator is a thermally actuated gas actuator.

23²² 46. (New) The device of claim ~~23~~²², wherein the heat source is a resistive heater.

13 46. (New) The device of claim ~~14~~⁶, wherein the valve is thermally actuated.

14 56. (New) The device of claim ~~46~~¹³, wherein the valve comprises a thermally responsive substance.

12 51. (New) The device of claim ~~14~~⁶, wherein the valve is reversible between an open and a closed state.